III.

CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY.

XI. — ON THE CARPOLOGIC STRUCTURE AND DEVELOPMENT OF THE COLLEMACEÆ AND ALLIED GROUPS.

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Of the many questions which, during the last half-century, have aroused more than a passing interest among mycologists, none have received more attention or excited more controversy than those relating to the nature and development of lichens. Such questions naturally present themselves under two phases, — the anatomy of the thallus, including the nature and mutual relationship of its component parts, and the origin and development of the fructification. Inasmuch as it is chiefly under the latter phase that the subject is presented in the present paper, it will be necessary to trace but briefly the course of the investigations which have led to the conclusion now generally accepted with regard to the nature of the lichen-thallus.

As is well known, the thallus of lichens is composed mainly of two elements, — a mycelial weft of hyphæ similar to ordinary fungus-hyphæ, and roundish cells of a grass-green or bluish-green color, existing either in a unicellular condition, or united into groups or into moniliform series of various types. According to the views of Fries,* published in 1831, and accepted as conclusive from that time until the year 1869, these green cells, the so-called *gonidia*, "are on the one hand nourishing organs of the thallus, and on the other hand non-sexual reproductive organs, which develop into new individuals either before, or subsequent to, their separation from the mother plant." It is hardly necessary to state that this view expresses the explanation at that time accepted of the origin and function of the gonidia of lichens, and their aggregation into the so-called "soredia."

Again as late as 1860 we find that this view of the nature of the

* Fries, Lichenographia Europæa Reformata.

gonidia is held by Schwendener, and expressed by him even more explicitly. "The green cells or gonidia," he says, "are, as is well known, lateral buds of the hyphæ, and as such are to be compared with branches. But whereas the branches, by repeated cell-division in one direction, lengthen without limit, in the development of the gonidia there occurs as a rule but one division of the primary cell to form one basal and one apical cell. The latter becomes spherical, while the former undergoes no change, and forms a longer or shorter stalk-cell." *

The continuation of Schwendener's investigations on the lichen-thallus, in 1863, indicates no change of view, and he reiterates the opinion formerly expressed by him, that "in all cases the gonidia arise as lateral outgrowths of the hyphæ, appearing therefore as spherical, green, apical cells of short, lateral, two-celled branches." † It is not until the publication, in 1868, of the concluding part of Schwendener's investigations, that the views already hinted at by De Bary with regard to the gelatinous lichens, are brought forward with much prominence, and the views held previously are seriously questioned. Speaking of the recently implied parasitic relationship between certain unicellular algæ and investing fungus-hyphæ to form a compound organism known as a lichen, Schwendener says: "Since the possibility of such a condition, and in some cases its probability, is no longer a matter of doubt, the question arises whether all lichens do not arise in the same manner." ‡

Finally, in the following year, the same observer, § breaking free from all tradition and preconceived ideas, boldly states his conclusion, based on new and more careful research, that lichens, so far from forming a group of autonomous organisms, are simply fungi parasitic upon algæ; that there exists no form of genetic connection between the two elements of the thallus; and that the compound organisms known as lichens, resulting from this peculiar condition of parasitism, are characterized by a form of fructification and development similar to that seen in many of the ascomycetous fungi. The bitter opposition which this assertion at once excited on the part of lichenologists was met by supporting arguments quite as vehement, but it is unnecessary to follow this discussion in detail here. It has been most ably

* Schwendener, Untersuchungen über den Flechtenthallus. Nägeli's Beiträge, Heft II. p. 125.

† Ibid., Heft III. p. 133.

‡ Ibid., Heft IV. p. 195.

§ Schwendener, "Die Algentypen der Flechtengonidien." See also Schwendener, "Erörterungen zur Gonidienfrage." Flora, May, 1872.

summarized in a series of papers published in the Quarterly Journal of Microscopical Science,* and to those papers the reader is referred for further information upon the subject. This summary closes with a brief account of Treub's experiments of 1873 in the culture of lichen-spores in the presence of unicellular algæ, and presents in a concise form all the evidence which conspired to establish beyond all controversy the first of Schwendener's hypotheses, that a lichen is a compound organism consisting of a fungus parasitic upon a living unicellular or filamentous alga. The grounds for this hypothesis were originally merely the remarkable similarity in outward appearance which was seen to exist between the gonidia of lichens and certain of the lower algæ, but the proof of the identity of these two similar organisms was to be found only from simple and synthetic cultures. As early as 1867, Famintzin and Barenetzky † had published the results obtained by them from the culture of the chlorophyllaceous gonidia of Physcia, Evernia, and Cladonia, which showed that from these gonidia were produced unicellular algæ identical in form and method of reproduction with Nägeli's genus Cystococcus; while from cultures made by Itzigsohn,‡ about the same time, of the gonidia of Peltigera, were produced *Glœocapsa monococca*, Kütz., and *Polycoccus punctiformis*, Kütz. Later, Woronin § confirmed these results by proving that from the zoöspores emitted by the gonidia of *Parmelia pulverulenta*, Ach., were produced algæ identical with those gonidia. In 1871 the Schwendener hypothesis received further confirmation from the experiments of Reess,‖ who sowed the spores of Collema on Nostoc colonies, and produced what might easily be considered a mature Collemaceous lichen, and two years later appeared the results of Bornet's¶ experiments in more than sixty genera of lichens, from which he drew the following conclusions: — 1. Every lichen gonidium may be referred to some definite species of alga. 2. The relations of the hyphæ and gonidia are such that neither could have arisen from the other, and the theory of parasitism alone can explain them.

It was reserved however for Treub, by the synthetic culture of

* W. Archer, Quart. Jour. Micr. Sci., Vol. XIII. p. 217, and Vol. XIV. p. 115.

† Famintzin and Barenetzky, Mémoires de l'Académie Impériale des Sciences de St. Pétersbourg, Sér. VII. Tom. XI. No. 9. Botanische Zeitung, 1867, p. 169.

‡ Itzigsohn. Bulletin de l'Academie Impériale des Sciences de St. Pétersbourg, Tom. VI. Botanische Zeitung, 1868, p. 185.

§ Woronin, Annales des Sciences Naturelles, Sér. V. Tom. XVI.

‖ Reess, Monatsb. d. K. Akad. d. Wiss. zu Berlin, 1871.

¶ Bornet, "Sur les Gonidies des Lichens," Comptes Rendus, Tom. LXXIV. No. 12.

lichen-spores in the presence of Cystococcus freed from another species of lichen, and for Alfred Möller by the successful culture of lichen-spores alone in a nutritive fluid, to settle the whole matter conclusively as far as the species cultivated were concerned. Möller's cultures, the preliminary results of which were published in 1887,* included fifteen species, representing ten genera of crustaceous lichens, and established the following points: "From a lichen-spore a fully differentiated thallus can be grown, the development of which can be followed on a glass slide without the use of an opaque substratum. Gonidia never appear in such a thallus provided that the culture-method employed secures complete isolation. Experiments on the germination of lichen-spermatia led to like results. A large number of spermatia germinated like other conidia, the resulting mycelium and thalline body could not be distinguished from those developed from an ascospore of the corresponding lichen, and, like it, developed new spermogonia whose spermatia corresponded to those originally sown."

Finally, the discussion on these lines was concluded by the results, published in 1889,† of Bonnier's careful synthetic cultures, extending over a continuous period of three years, during which time, by spore cultures of a large number of heteromeric lichens in the presence of algæ procured from pure cultures of previously determined algæ, he produced a number of typical, and in some cases fruiting lichens. The reader's attention, however, must be drawn to the fact that as yet we have heard nothing from Möller on the subject of the homœomeric lichens, and that Bonnier was unsuccessful in his experiments upon Collema and Ephebe.

Meanwhile, as botanists became convinced that Schwendener's first hypothesis with regard to the parasitic nature of the lichen fungus was an indisputable fact, attention came to be directed to other kindred topics of no less interest and importance. If it had not been definitely stated, it was at least tacitly assumed by most of the earlier mycologists, that lichens were sexual in their method of reproduction, and that the spermatia were the male organs. But we have seen that from these very spermatia Möller professes to have grown fully developed thalli, without the intervention of any female organ. If this is so, it is a fact which must militate very strongly against the view that the fruit of the corresponding lichens is in any respect sexual in

* Möller, "Ueber die Cultur Flechtenbildenen Ascomyceten ohne Algen," 1887.

† Bonnier, Annales des Sciences Naturelles, Sér. VII. Tom. IX., 1889. Revue générale de Botanique, I., 1889.

its origin, for it is certainly without analogy that any form of reproductive bodies should be at one and the same time male organs, and, as stated by Möller, "conidia with slightly weakened functions," unless indeed we consider as in any way analogous the supposed reversion to a vegetative condition of the pollinodium or fertilizing cell of certain Ascomycetes. In view of the fact that these spermatia or conidia possess the function of non-sexual reproduction to by no means the normal extent, it is certainly possible that they are to be considered as degraded forms, which by disuse have lost their former sexual function, a function which we find is retained by similar organs in other lichens. This view is not without analogy. It is certainly a significant fact, that, as will be seen later, the character of the spermatia as non-sexual reproductive bodies exists only in those lichens where no form of sexual reproduction has as yet been observed with certainty, and that, in the group of lichens where sexual reproduction exists, all the knowledge that we possess regarding the spermatia points to their sexual character. There was nothing approaching any certain knowledge on this point until 1877, when Stahl published his remarkable observations on certain homœomeric and heteromeric lichens.* A reference to his paper will show that of the species examined by Stahl all but three belong to the Collemaceæ, these three exceptions being members of the angiocarpic and gymnocarpic groups of the heteromeric lichens, about which very little of a definite nature is said. On the subject of the Collemaceæ, however, Stahl is much more explicit, and his observations, depending upon careful treatment and accurate observation, seem, if true, capable of satisfactory proof. In brief they are as follows: — Stahl has observed, embedded in the gelatinous substance of the thallus, peculiar hyphæ in the form of more or less definite knots or loose spiral coils. This coiled series of richly protoplasmic cells, designated as the "ascogonium," and corresponding to what is known in certain ascomycetous fungi as "Woronin's hypha," is continued upwards in a multicellular slender thread, reaching the surface of the thallus by piercing the investing cortex, and designated by Stahl as the "trichogyne," on account of its similarity in function to the organ of that name seen in certain of the Florideæ. With the projecting tip of this organ the spermatia come in contact, and adhere to its sticky surface. Although, owing to the small size of the spermatia, protoplasmic union between them and the tip of the trichogyne has not been observed, the subsequent changes

* Stahl, "Beiträge zur Entwicklungsgeschichte der Flechten." 1877.

in both trichogyne and ascogonium point to the probability of such a union. In the trichogyne these changes are seen in the progressive thickening of the septa from the tip inwards, while the cells of the ascogonium begin to multiply in number. The vegetative hyphæ in the neighborhood then proceed to branch profusely, enveloping the ascogonic cells, and finally putting out among and between the latter a series of parallel threads, arising perpendicular to the surface, and forming the first paraphyses. The asci arise solely as ultimate branches of the ascogonic cells, and thus the two systems forming the young hymenium are from the beginning quite separate and distinct from each other.

These facts have been observed in detail only in the Collemaceæ, and Stahl explains them on the ground that the coiled ascogonium is analogous to that seen in Eurotium and Sordaria both in form and in later development, the absence of the trichogyne in these forms being due to the suppression of motile male bodies and the introduction of fertilization by a process of fusion between neighboring specialized branches. He considers the trichogyne as quite analogous to the organ of the same name in the Florideæ, and from these considerations he draws the general conclusion that all lichens are probaby provided with a form of sexual reproduction analogous in some respects to the Florideæ, and in other respects to certain ascomycetous fungi. But such a generalization is at least premature when we consider that it is only in the peculiar group of the Collemaceæ that these analogies have been traced.

With this question concerning the sexuality of lichens there has recently been connected another of almost equal importance. In all ascomycetous fungi in which some type of sexual reproduction has been certainly observed there are also found to exist two separate systems of hyphæ in the fruit, — the ascogenous system, arising from the ascogonium, and consisting of asci only, and the enveloping system, arising from the hyphæ surrounding the ascogonium, and consisting of the paraphyses and the tissue enveloping the hymenium. This fact is so marked a feature of sexual reproduction in fungi, that it seems fair to conclude that it is characteristic of such reproduction. Both these points are brought out prominently by Stahl in his investigations on the Collemaceæ, but here again the generalizations made by Stahl, and almost tacitly accepted by many later observers, are certainly not to be accounted for on grounds of analogy. The lichens, exclusive of the hymenomycetous and gasteromycetous forms, are now considered generally to belong to the great class of

the Ascomycetes. It seems pertinent, therefore, to ask why we should expect to find throughout the lichens this highly differentiated form of reproduction alone. Should we not rather, arguing from analogy, expect to find many modifications of this type, starting with that seen in the Collemaceæ which connects that group more or less definitely with the Florideæ, and including in the series some, if not all, of the modifications existing in such ascomycetous fungi as Polystigma, in which a trichogyne, while present in the form of a prolongation of a coiled ascogonium, takes no share in the formation of asci, — Pyronema, in which fertilization is effected between the swollen tips of adjacent hyphal branches by means of a tube put out from one of the tips (the male cell or antheridium) which fuses with the tip of the female cell or ascogonium, — Podosphæra, Erysiphe, Eurotium, and Ascobolus, where a coiled ascogonium is fertilized directly without the intervention of a trichogyne, by protoplasmic union between its tip and that of a specialized branch arising in its neighborhood, — and, finally, the sclerotia-forming Pezizæ and the Morchellæ, in which, with the disappearance of the carpogonium, the last traces of sexual organs are lost entirely and reproduction becomes purely a vegetative process?

The present paper embodies the results which I have obtained from the careful examination of certain species of lichens presumably most nearly related to the Collemaceæ, with the twofold object of either proving or disproving, in the lichens examined, the existence at any time of two separate systems of hyphæ in the fruit, and the occurrence of some form of sexual reproduction. I was led to a discussion of this topic by a paper recently published in "Flora,"* which deals with the same questions. The writer, after examining nine representatives of the genera Anaptychia, Ramalina, Physcia, Parmelia, Xanthoria, Placodium, Lecanora, and Lecidella, states his conclusions as follows: —

1. In all species examined, the ascus system and the enveloping system are separate and distinct from each other.

2. In all there is great similarity in the development of the apothecium to that in the Collemaceæ as observed by Stahl.

The first proposition rests on the presumed fact that there exists an ascogonium from which arise asci only, but Lindau presents no direct proof of this. The second proposition, even if we accept all Lindau's statements, states too much, since there often exists a marked discrepancy between his results and those recorded by Stahl. One point

* Lindau, "Ueber die Anlage und Entwicklung einiger Flechtenapothecien," Flora, 1888, p. 451.

observed by Stahl, and used by him very largely as a proof of sexuality, consists in the changes which take place in the trichogyne subsequent to the attachment of one or more spermatia to its tip. This point has not been observed at all by Lindau; in fact, in one or two cases he fails to observe the trichogyne at all, and merely assumes its existence from analogy. According to Lindau the trichogyne disappears entirely from older primordia in which the ascogonium has become completely enveloped by branches of the neighboring hyphæ, whereas Stahl by his figures of *Physma compactum*, Mass., shows very plainly that the changes in the trichogyne do not take place until the ascogonium is already enveloped by the hyphæ. Furthermore, Lindau confesses that he "is unable to see the bridge-like connection of which Stahl speaks between the spermatia and the trichogyne, although in this case (*Anaptychia ciliaris*) the size of the spermatia might make it easy to see any union if it existed." Stahl bases his distinction between the trichogyne and the ordinary vegetative hyphæ largely on the fact that the former never branch; on the other hand, Lindau states, in one place, that the primordia may be arrested in their development and then revert to the vegetative condition, while in others the young ascogonium bifurcates, and each branch becomes a coiled ascogonium, never reverting to the vegetative condition, the latter point being used as a proof that the ascus system is distinct from the enveloping system. There are many other points in the structure of the ascogonium and its share in apothecial formation, which differ so widely not only from Stahl's observations, but among themselves, that it seems almost incredible that they should occur in groups so nearly related otherwise as those examined by Lindau. We are at least justified in regarding the method of reproduction in the heteromeric lichens as still unsettled, and in continuing careful and systematic investigations upon their structure, until more light is thrown upon it. Taking it for granted that in the groups more nearly related in structure and habit to the Collemaceæ we should find, if anywhere, traces of a form of reproduction found in that group, and a marked separation of the ascus system from the enveloping system, I began my investigations upon members of the family Peltigerei, genus Sticta.

The method of investigation followed has been practically the same in all cases. To procure the youngest stages of apothecial development, I made a large number of sections by hand in the neighborhood of the youngest apothecia visible to the naked eye, and seldom failed to secure the desired stages. This method of making sections I have found preferable to the use of the microtome, inasmuch as the process

of embedding in paraffine seems ill adapted to the lichen-tissue. In those lichens which are invested with a tough cortex, it is wellnigh impossible to secure the requisite degree of infiltration with the reagents usually employed, and the attempt to use the microtome reveals the fact that the paraffine has penetrated very slightly, if at all, into the inner tissue of the thallus, while the gelatinous lichens are, from their very nature, liable to much distortion during the process of embedding.

To ascertain the mutual relationship existing between the asci and paraphyses, I have again had recourse to thin sections of young apothecia cut by hand, selecting for that purpose apothecia in which the young asci were just visible among the more fully developed paraphyses. By treatment of such sections with various clearing and staining reagents, and subsequently crushing them under the cover glass, the origin of both asci and paraphyses may be readily seen.

Sticta anthraspis, Ach.

The medulla of this lichen consists of a loosely interwoven layer of comparatively thick-walled, sparingly septate, hyaline hyphæ. Owing to the slight degree of complexity in the tissue, the generally regular course and the large size of these hyphæ, they may be followed separately in their course, often for a long distance, especially after treatment with alcohol to expel all air from the section, and subsequently with dilute potassic hydrate. The youngest stages of the apothecia can thus be made out with considerable accuracy. The surface of this lichen presents a peculiar pitted appearance, due to an irregular network of raised veins or ribs, which in section present the normal thalline structure. It is upon these ribs almost exclusively that the spermogonia and apothecia are borne. The former appear to the naked eye as slight elevations on the ribs, pierced in the centre by a minute dark brown pore. They arise in the upper part of the medullary layer as small spherical knots of hyphæ. As one of these knots increases in size, it becomes more and more dense, and soon reaches the outlying groups of the algal layer. Hitherto the structure of the knot has been more or less clear; now, however, it becomes very dense, and from spherical becomes ovoid, the pointed end being directed upwards. As it grows, it pushes aside the groups of algæ composing the gonidial zone, crowding them together on all sides, and frequently pushing them well back into the medullary layer. As soon as the apex reaches the thick parenchymatous cortex at one point, the cells of the latter at this point apparently increase in size, this appearance being due to the

breaking down and absorption of the membranes between adjacent cells. This lysigenetic process is continued until a pore is formed piercing the cortex, and occupying the centre of a slight elevation on the surface caused by the expansion and upward growth of the young spermogonium. Meanwhile, before the formation of the pore, the central part of the spermogonium has become hollow by absorption, and the space thus left becomes, in the mature spermogonium, almost filled with stout, branched, multicellular branches of the hyphæ forming the wall; on the joints of these — the so-called arthrosterigmata — are borne the minute spermatia in vast numbers. As yet I have been unable to secure the youngest stages in the development of the apothecium, but much may be inferred from stages already rather advanced. To the naked eye these appear as slight elevations on the ribs, closely resembling the mature spermogonia, except that they are a trifle larger, of a dark brown color, and present no trace of an orifice at the top. In section they are seen to be spherical masses of very delicate densely interwoven hyphæ about 0.3 mm. in diameter, occupying the upper part of the medullary layer, displacing the algæ immediately above them, and covered by the brown cortex. The lower half of one of these hyphal knots is permanent, and retains its dense hyphal structnre, but the upper half soon becomes disorganized, the hyphæ composing it being partially absorbed, leaving a hemispherical cavity, from the upper part of which the broken ends of the original hyphæ may be seen hanging down into the cavity. Meanwhile, as the cavity forms, the permanent tissue beneath gives rise to a series of delicate parallel threads which grow upwards into the cavity, and we thus have formed a young hymenium imposed upon a dense subhymenial layer, which owes its origin to a copious branching of ordinary medullary hyphæ. Treatment with iodine, or with chloro-iodide of zinc, has the effect of coloring both the subhymenial layer and the young paraphyses a deep yellow, darker and more pronounced than the color imparted to the ordinary hyphæ of the medulla. But no blue coloration is as yet visible in any part of the structure, nor is there any sign of a trichogyne even in this early stage, or of anything which might pass for an ascogenous system of cells in the subhymenial layer. The young hymenium increases rapidly in size by the interposition of new paraphyses as well as by the branching of those already formed, until one of two things occurs: either the increase in area of the hymenium takes place over its whole extent, until the tension upon the cortex becomes too great, and the latter is ruptured over the whole disk; or else, in cases where the apothecium arises in the gonidial layer imme-

diately under the cortex, the disk has no time to enlarge sufficiently to rupture the cortex, as seen in the first case, but the paraphyses first formed pierce it at one point, and, acting like a protruding cone, force apart its tissue. In either case, the expansion and upward growth of the hymenium soon cause it to appear on the surface of the thallus in its normal disk-like form, the elevated border or exciple being formed partially by the marginal paraphyses, but almost entirely by the cortex, which by the elongation of its cells in the direction of the course of the hyphæ, i. e. perpendicular to the surface, becomes much thickened.

The algæ composing the gonidial layer, when freed from the thallus by maceration, are seen to exhibit the bluish-green color of the Cyanophyceæ, and form almost unaltered colonies of Chroococcus, Næg. The density of the spherical mass of hyphæ forming the young apothecium excludes the algæ from it, and with the growth of the former, and the subsequent rupture of the cortex, the algæ are forced aside, so that the mature apothecium consists only of a dense subhymenial layer arising from and resting upon the normal medullary hyphæ, and giving rise above to the hymenium, the whole being surrounded by the upturned and thickened cortex. It occasionally happens that here and there an algal colony is enclosed and carried up in the lower tissue of the apothecium; but the conditions are unfavorable to its growth and multiplication, it soon dies, the contents of the cells disintegrate, and the shrunken hyaline membranes alone remain. Not until the young apothecium has burst through the cortex, and has attained a diameter of almost or quite 1 mm., do the asci appear. They can then be distinguished by treatment with iodine, and are seen to be scattered very sparingly through the whole hymenium. The iodine brings out several important features. First, the asci alone are colored, and the blue color does not extend appreciably into the subhymenial tissue. The ascogenous cells then, if they exist, do not partake of the chemical nature of the ascus membranes as exhibited by a blue coloration with iodine. Secondly, the whole subhymenial layer is colored a deeper brown than the surrounding tissue, and this coloration is homogeneous throughout the layer. The ascogenous cells then, if they exist, do not differ materially in size or in their reaction with iodine from the cells which give rise to the paraphyses. These apparent facts are borne out by further investigation. If a thin median section through a young apothecium in which asci are just beginning to appear, be treated first with alcohol to expel all trace of air, and then with dilute potassic hydrate, it becomes very transparent, and a dilute tincture of

iodine brings the asci into clear relief. By pressure upon the cover-glass the elements of the hymenium may now be separated, and if the treatment with potassic hydrate and then with tincture of iodine be repeated, and the superfluous iodine washed out with water, the asci appear colored a deep blue, and the paraphyses yellow to brown, and both may be traced to their origin. It is then seen that the subhymenial tissue consists of septate hyphæ a trifle smaller than the medullary hyphæ and much distorted by mutual pressure. Even when, as is often the case, the process of clearing has rendered the cell membranes invisible, the continuity of the deeply stained protoplasm shows that both asci and paraphyses arise from the same hyphæ. Sometimes a single ascus is seen to be surrounded by a tuft of paraphyses springing from its basal cell, and sometimes an ascus arises as a branch from the basal cell of a paraphysis. (Plate I. Figs. 1–3.) But however the two systems are related in position, they have a common origin from one and the same set of hyphæ. This fact seems to militate conclusively against the theory of sexuality, especially when taken in connection with the apparent absence of ascogonic cells, either within the thallus in the neighborhood of young apothecia, or in the tissue of the youngest apothecia themselves, and the absence, as far as I have observed, of trichogyne tips projecting above the surface.

Sticta amplissima, (Scop.) Mass.

In this species the general structure of the thallus is essentially the same as in *S. anthraspis*, the slight modifications being due to the different nature of the algæ composing the gonidial layer. Whereas in *S. anthraspis* they were united into colonies by gelatinous sheaths, here they are seen to be very small, unicellular, and bright grass-green in color, and belong therefore to the chlorophyllaceous genus Protococcus, Ag. The gonidial layer is consequently much more dense, and the medullary layer therefore more closely interwoven, and its hyphæ more irregular in their course, than was the case in *S. anthraspis*. Let us pass at once, then, to considerations regarding the formation of the fruit. It is not infrequent to find a thallus on which there is no trace of an apothecium, but which is covered with spermogonia in all stages of development visible to the naked eye. Thin sections almost anywhere in such a thallus give the youngest stages of the latter. In places there are seen, with the low power, occupying the lower part of the gonidial layer, pale oval spots. With a higher power, such a spot is resolved into a dense mass of fine branches of the medullary hyphæ, not enclosing gonidia, and therefore appearing pale

in comparison with the surrounding tissue. The cells composing these hyphæ are copiously filled with small drops of oily matter. The mass when first plainly visible measures about 68 μ in diameter. In a slightly later stage the mass has become lengthened considerably in a direction perpendicular to the surface, and is now flask-shaped, the neck — a solid cylindrical shaft of hyphæ — being almost as wide as the spherical mass below, and extending through the gonidial layer very nearly to the surface. Later stages show that this mass, the young spermogonium, now increases very rapidly in size, the centre however occupying about the same depth in the thallus. From this it follows that the mature spermogonium occupies nearly the full depth of the thallus, extending as far towards the lower as towards the upper surface. As the young spermogonium grows, the hyphæ composing what I have called the neck become more and more merged in the spherical part of the spermogonium, until at maturity the latter possesses no real neck, it is no longer flask-shaped but spherical, and its upper surface is so slightly beneath the surface of the thallus that the absorption of cell-membranes and the formation thus of a minute pore, suffices to establish a connection between the interior of the now hollow spermogonium and the outer air. As in *S. anthraspis*, the mature spermogonium is almost filled with jointed arthrosterigmata growing out from the walls, and bearing countless rather large oval spermatia.

I have said that some thalli produce spermogonia almost exclusively; as might be inferred, others are found covered with apothecia only. Near the centre they occur so plentifully that they almost hide the thalline surface; toward the margin they occur more sparingly and in younger stages, while near the extreme edge they are wanting, and a very few scattered spermogonia are found. If thin sections are made in that part of the thallus where apothecia in great numbers are just visible with the hand-lens as minute elevations of the thalline surface, we find in considerable numbers, in the lower part of the gonidial layer, small, irregularly spherical masses of hyphæ, displacing the algæ. These hyphæ are filled with small oil-drops, the masses measure from 60 to 70 μ in diameter, and, in fact, both in position in the thallus and in general appearance, they remind us of what we found to be the earliest stages of spermogonia. Closer examination, however, shows points of difference. In the first place, they occur in a thallus almost destitute of spermogonia; the masses are also more decidedly spherical, nor is there ever seen in later stages the lengthening upwards which always occurred in the young spermogonia; and, finally, the

stages in their growth may readily be followed in a series of sections closing with the mature apothecium.

Let us take one of the smallest of these masses. It measures 0.65 mm. in diameter, and of course possesses no trace of asci or paraphyses. Potassic hydrate makes the mass perfectly transparent, but the most careful search in many specimens fails to reveal anything like an ascogonium, or any cells larger or in any respect different from the vegetative hyphæ which gave rise to the mass. Furthermore, although this is presumably the youngest visible stage of the apothecium, and the disappearance of the trichogyne is always said to be the mark of a comparatively advanced stage, no coiled hypha is seen, and no vestige of a trichogyne tip is to be found projecting above the smooth, unbroken surface of the cortex. Treatment with tincture of iodine, or with a solution of iodine and potassic iodide, also fails to bring out any hint of the existence of an ascogonic system. Very often in the external part of these masses are seen, when treated with iodine, irregularly shaped bodies of a dark brown color which might easily be mistaken for the large, deeply-stained cells of an ascogonic hypha. Careful focusing, however, or if that fails, crushing of the section, shows that they are nothing more than algæ which have become separated from the gonidial layer, enclosed in the hyphæ forming the apothecial primordium, and much distorted by the pressure of these hyphæ.

The next stage in the development shows that the primordium has increased rapidly in size. It now measures from 0.18 to 0.2 mm. in diameter; but with the exception of this increased size, and a rather decided flattening of its lower part, there is no marked change. The mass is still capable of being resolved into a confused and tangled aggregation of hyphæ, but they are beginning to show a tendency to grow in a general direction more or less perpendicular to the surface. Neither ascogonium nor trichogyne has as yet appeared. One thing, however, must be borne in mind. The hyphæ composing the primordium have by this time become, by mutual pressure, rather smaller and considerably more distorted than the medullary hyphæ, and are therefore much dissimilar from them in appearance. If now a piece of an ordinary medullary hypha by any chance overlies our section in a definite position, or if, by focusing too deeply, we come upon one of such parallel hyphæ, it will present the appearance of a large-celled hypha embedded in the primordium, and might easily be mistaken for an ascogenous hypha. No mistake, however, can easily be made with regard to the presence or absence of a trichogyne, and

in none of the many sections examined by me has any trace of such an organ appeared.

We next find that the upper half of the young apothecium has disappeared, presenting the appearance of having been torn apart by the cessation of growth below, and from the upper part of the cavity thus formed are seen suspended the remnants of the hyphæ. The lower, flattened surface of the cavity is now seen to be composed of a dense layer of young paraphyses. The gonidial layer has been raised up over the young apothecium, only traces of it appearing below in the shape of a few separate algæ, or small groups of them, which have been embedded in the lower part of the young apothecium and remain there. The first evidence of the coming exposure of the hymenium, is seen in the looşening of a broad, wedge-shaped portion of the algal layer and the cortex immediately above the young hymenium, as though by the expansion of the latter it were to be thrown off bodily. The later development is like that described in the case of *S. anthraspis*. Before the cortex is ruptured, and the hymenium exposed, the asci begin to appear, at first sparsely throughout the hymenium, but soon in greater numbers; the tissue covering the disk is thrown off, and the hymenium, surrounded by a thick, thalline exciple, appears on the surface. To determine the origin of asci and paraphyses the same method of treatment may be employed as before; and here again the asci alone are colored blue, the coloration not extending into the hymenium. No more than in the case of *S. anthraspis* is it possible to find two separate systems of hyphæ in the subhymenial tissue. The figures (Plate I. Figs. 4–6) show a variety of forms by which the relation between the asci and paraphyses is established.

Nephroma tomentosum, (Hoffm.) Krb.

The genus Nephroma, as already pointed out by Schwendener* and also by Tulasne,† forms a connecting link between the genera Sticta and Peltigera. To quote from the author first named, "Whereas the peltate, borderless apothecia emphasize the affinity of this genus (Nephroma) to Peltigera and Solorina, the anatomical structure of the thallus presents such a perfect agreement with that of Sticta, that it is impossible to find a single characteristic which cannot also be found in one or another species of the circle of Sticta forms." ‡

* Schwendener, Nägeli's Beiträge, Heft III. p. 166.

† Tulasne, Mém. Lich., pp. 20 and 145.

‡ Schwendener, *loc. cit.*, p. 173.

In the thalline structure of the species before us, we find so marked a resemblance to that of *Sticta anthraspis*, that we may pass at once to a brief consideration of the origin and growth of the apothecium. As is well known, the large, peltate apothecia occupy rather sparingly the lower surface of the extended lobes, and it is a matter of some difficulty to obtain the earliest stages in the development of the fruit. By making careful longitudinal sections, however, through the tips of the thallus lobes, I was enabled to see more or less plainly the origin, and to trace the later development, of the apothecia. The first sign of the coming apothecium is the appearance of a small spherical knot of hyphæ arising from the medullary hyphæ, and occupying a position immediately below the algal layer, which, at the margin of the thallus, is very thin and composed of only scattered colonies. The primordium never occupies the exact tip of the margin in section, but inclines more or less toward the lower surface, sometimes, indeed, arising from the medullary layer beneath the lower cortex and at some distance from the margin. The few algal colonies which do occur between the primordium and the lower surface of the thallus are pushed aside by the growth of the former, which then remains covered only by the cortex. Before the cortex is disintegrated or ruptured, the young paraphyses arise as perpendicular branches of the knot of hyphæ. No part of the knot, or of the paraphyses arising from it, is colored blue by iodine, but the whole gives homogeneously the ordinary protoplasmic reaction. The growth of the young hymenium by the interposition of new paraphyses is equally rapid over the whole area, so that the cortex covering it is stretched more and more, and finally ruptured simultaneously over the whole disk. The hymenium then increases even more rapidly than before, relieved of the tension of the cortex, and if it does not already occupy the lower surface of the thallus, the addition of paraphyses is more in that direction, and by the time the young apothecium is distinctly visible to the naked eye, it is seen to occupy entirely that position. Often four or five young apothecia arise in such close proximity to one another, that, in the ordinary course of growth, the intervening tissue is broken down, and one large apothecium results.

A section of the young apothecium exhibits a margin of extremely simple structure, if indeed it can be called a margin at all. By reason of the pressure exerted by the growing hymenium upon the investing cortex, the cells of the latter are lengthened considerably in the direction of hyphal growth, i. e. perpendicular to the surface ; those hyphæ of the cortex which are in direct contact with the paraphyses become distinguishable from them only by their greater diameter, and the

margin here equals in thickness the height of the paraphyses. They retain more and more their original dimensions as they recede from the hymenium, until they are found to present entirely their normal appearance as cortical hyphæ. Until the apothecium is nearly half the size which it attains at maturity, the asci remain invisible, being enveloped and covered by the semi-gelatinous membranes of the paraphyses; but the same treatment which was made use of before serves here also to bring the asci into relief. I have found it advisable, however, in all cases where the membranes of the paraphyses are of so gelatinous a character as in this species, to avoid the use of any alcoholic reagent, inasmuch as the alcohol induces a contraction and hardening of the tissue which effectually prevent subsequent maceration. If it is found necessary to stain the asci, an aqueous solution of iodine and potassic iodide may be used. Subsequent maceration shows that the subhymenial layer consists of branches of the medullary hyphæ so densely woven together that a pseudo-parenchymatous tissue results, the cells of which, although in places they still retain a linear arrangement, have as a rule become swollen and distorted by mutual pressure, and are rather larger and more nearly isodiametric than the original hyphal cells which they represent. From this tissue both asci and paraphyses arise indiscriminately, the paraphyses first, and later the asci, pushing up between them. There is no visible differentiation of the subhymenial tissue into ascogenous and paraphyses-bearing hyphæ; both arise from one and the same tissue of medullary hyphæ, modified, it is true, but not into two dissimilar systems. (Plate I. Figs. 9–11.)

Peltigera, (Willd., Hoffm.) Fée.

Of the genus Peltigera I have examined but one species, *P. polydactyla*, (Neck.) Hoffm., and very little need be said with regard to it, owing to the similarity existing between it and the other genera previously studied. The thalline structure resembles very closely that of *S. amplissima*, but approaches more nearly still perhaps that of *N. tomentosum*, the only important anatomical difference being the absence of the cortex on the lower surface. Peltigera differs carpologically from Nephroma only in the fact that whereas in the latter, as we have seen, the apothecial primordium arises near the lower algal zone, and when mature occupies the lower surface of the thallus lobe, the primordium in Peltigera arises just below the upper algal zone, and, its growth being exclusively toward the upper surface, the mature apothecium occupies that surface. I have been unable in Peltigera, as in Nephroma, to find any trace of a carpogonic apparatus. The origin

and development of the apothecium seem to be purely vegetative processes, nor, as far as I have observed, is there any distinction, at any stage in the development, between the ascogenous and the enveloping systems of hyphæ. Owing to the gelatinous quality of the membranes of the paraphyses, and the frequent fusion of their tips, it is a matter of much difficulty to separate the elements of the hymenium and trace them to their origin. This may be done, however, as in Nephroma, by careful crushing, after treatment with potassic hydrate and then with an aqueous solution of iodine and potassic iodide. The blue coloration of the asci is as permanent as with the tincture of iodine. The coloration of the protoplasm, however, is readily washed out by the subsequent treatment with water, though this will be found to be no particular disadvantage. (Plate I. Figs. 7, 8.)

Thus far we have considered types of genera which present on the whole the structural characteristics of the heteromeric, foliaceous lichens. But we have already seen tendencies toward another type. In *Nephroma tomentosum* we have seen that the algæ forming the host are no longer of the grass-green, unicellular type, but are bluish-green cells united into colonies, and belonging evidently to the phycochromaceous genus Glœocapsa, Näg., and the same is true of many species of the genus Peltigera. In fact, the family to which this latter genus belongs presents in a very marked degree this transitional character with respect to the gonidia. Three of the five genera included in it by Tuckerman are characterized by bluish-green algæ in one group of species and grass-green algæ in another group; another genus possesses both types of algæ in one and the same species, while the fifth genus is parasitic on bluish-green algæ alone. Coming to the family Pannariei, however, we find this transitional feature even more strongly marked, one only of the four genera being in part parasitic on grass-green algæ. Furthermore, we shall see that in this genus we begin to lose sight of the Chroococcus and Glœocapsa types of algæ, and find a marked approach toward the homœomeric lichens in the occurrence of a filamentous type of alga. Finally, in the loss of the lower cortex in the genus Peltigera, we must recognize an added link in the chain of evidence connecting the heteromeric with the homœomeric lichens.

PANNARIEI.

In this family, presenting as it does, so many structural characteristics which are emphasized in the true gelatinous lichens, we might fairly expect to find traces at least of the type of sexual reproduction presumably characteristic of the Collemaceous lichens.

Heppia, Näg.

The first member of this great family which I have studied accurately is the genus Heppia, represented in North America by two species, *H. Despreauxii*, (Mont.) Tuck. [*H. urceolata*, (Näg.) Hepp, *H. adglutinata*, (Krmph.) Mass.], and *H. polyspora*, Tuck. The former presents many characteristics which adapt it peculiarly to anatomical study. In thin sections of the small thallus lobes we are at once struck by the fact that the fungus hyphæ, instead of pursuing a general direction parallel to the substratum, and forming a more or less closely interwoven tissue, occupy a position perpendicular to the substratum. This peculiarity is accentuated, and doubtless induced, by the position of the gonidial algæ. Instead of being scattered singly or in groups, they present a more or less pronounced linear arrangement parallel with the hyphæ. The hyphæ themselves are extraordinarily large, measuring near the substratum 4 μ in diameter, and increasing rapidly in size until at the upper surface the individual cells measure 18.8 $\mu \times 11.3\ \mu$. The cell walls are very thin, and the hyphæ lie closely packed together, so that the hyphal character of the thallus is lost except near the substratum, where the separate hyphæ are smaller and the texture looser. The cortex, when present at all, shows a marked change from that seen in the preceding forms, being reduced on the lower surface to a single layer of rather large thick-walled cells of a brownish color, while the upper surface is destitute of a cortex, unless we accept as our idea of a true cortex Schwendener's statement,* that "only a few layers of cells (sometimes only one) are destitute of gonidia, and may therefore be regarded as a cortex." The superficial cells, it is true, are covered by a delicate, hyaline, structureless layer, and the superficial cell wall is slightly thickened and colored brownish, but the differentiation of the cells composing this layer from the cells below is hardly sufficient to warrant our regarding the former as a true cortex. The gonidial and medullary layers are as little to be distinguished as distinct zones, inasmuch as the algæ occupy nearly

* Schwendener, *loc. cit.*, p. 178.

the full depth of the thallus; in fact, the whole character of the thallus would lead us to regard Heppia as one of the terminal members of a transitional series of forms connecting the heteromeric and homœomeric groups.*

I have before referred to certain peculiarities exhibited by the algæ of this lichen. Their peculiar arrangement, brought out most plainly with the low power, seems to indicate that we have to do with an originally filamentous alga, the elements of which have become partially displaced and altered in form by the encroaching hyphæ. This view is confirmed when, on crushing a section of the thallus, we are able to separate short chains consisting of four or five large, bluish-green cells. The analogy between these chains or filaments and the filamentous alga Scytonema, Ag., was first pointed out by Bornet,† and an examination of the species before us leaves no doubt of the truth of the analogy, although I have not as yet found the filaments still invested by the gelatinous sheath characteristic of Scytonema. Such filaments, however, do occur in all cases, upon the substratum under and around the thallus-lobes.

The origin and development of both spermogonia and apothecia differ only in minor particulars from what has already been observed and described in other lichens. The former are extremely small, a fact which, taken in connection with their rather infrequent occurrence and inconspicuous character, might cause them to be overlooked. Careful sections, however, near the edges of the thallus-lobes bring to light, generally just below the median line of the section, small hyaline areas in the midst of the surrounding algæ which on further examination are resolved into ovoid masses of delicate interwoven hyphæ. The later stages of development may be readily followed, and, being similar to those already described, are too familiar to need repetition here. By the time the apex of the young spermogonium has reached the surface, the central portion of the mass has been absorbed, and a pore is formed by a schizogenetic process, the hyphæ forming the short neck separating in the centre and forcing apart the parallel hyphæ surrounding them. The primordia of the apothecia resemble very closely the young spermogonia, except in size. When still distinguishable in the thalline tissue as only a dense, spherical mass of delicate, hyphal branches, they are already more than twice the size of the spermogonia of the corresponding stage of development. At this early

* Cf. Koerber, Parerga Lich., p. 26.

† Bornet, Ann. des Sci. Nat., Sér. V., Vol. XVII.

stage there is no visible sign of ascogonium or trichogyne, or indeed of anything which might indicate in the least degree any sexual origin of the apothecium. The apothecium originates rather above than below the median line of the section, so that there exist beneath it considerable numbers of gonidia. As the apothecium grows, it encroaches more and more, not only on the overlying tissue, but also on that below, the result being that the algæ occupying the latter are pressed together downward, and finally form a dense compact layer beneath the apothecium. If we accept the view that the presence of the algæ may induce a more vigorous growth in the hyphæ, this fact of the accumulation of the algæ may account for the origin of the cortex which is always found covering the lower surface immediately beneath the mature apothecium. There is one point in the development of the apothecium to which attention must be drawn. The young paraphyses, arising very early as perpendicular branches of the upper part of the primordium, attain their full length before the cortex is ruptured. The subsequent growth of the hymenium is entirely in area, and by this means the cortex is soon ruptured; but whereas in the other cases examined the later growth of the paraphyses and subhymenial tissue raises the hymenium considerably above the surface, in Heppia the upward growth has ceased by the time the cortex is ruptured, and the apothecium remains sunk in the thallus and separated from the thalline tissue only by a thin layer, one or two cells in thickness, formed of the compressed thalline hyphæ. Not until the hymenium is nearly or quite exposed do the asci appear, rising from the dense subhymenial tissue and pushing up between the paraphyses. Meanwhile the treatment followed in other cases fails to show the presence of any differentiated cells in the subhymenial layer. I have been unable to see any trace of a trichogyne, and maceration after treatment with potassic hydrate shows a relation existing between the asci and paraphyses identical with that seen in the other genera studied. (Plate IV. Figs. 23–25.)

Pannaria molybdea, (Pers.) Tuck.

In this lichen we find a very peculiar thalline structure. The lower part of the thallus is formed of a dense layer of hyphæ running parallel with the substratum, or more or less obliquely to it. This layer is continuous around the edge of the thallus, and forms upon the upper surface a layer which is thinner and more strikingly parenchymatous than the layer forming the lower surface. There is thus left a space between these two parallel layers which is filled by a much looser tissue of hyphæ arising from the lower layer and growing upwards to

become merged in the upper layer. The position of the algæ is in conformity with this peculiar structure. Scattered very sparsely through the dense lower layer, and corresponding in their course to that of the hyphæ in which they are embedded, are seen long filaments of bluish-green cells. In the loose tissue forming the central part of the thallus these filaments are shorter, and the cells composing them are considerably larger, than those of the scattered filaments below, (since the latter are decidedly compressed by the density of the surrounding tissue,) and their general position is perpendicular to the surface. This structure of the thallus must be fully understood in order to enable us rightly to interpret the stages in the development of the fruit. The spermogonia arise in the lower part of the loose central tissue, and present in respect to their origin and development no peculiarities worthy of notice. The origin of the apothecia is more peculiar, and more difficult to follow. The first step takes place in the form of an active branching, in a purely vegetative manner, of the hyphæ composing the upper part of the dense layer of parallel hyphæ already noted as bounding the thallus on its lower surface. This tendency to increased activity in growth, starting at one point, is transmitted to the loose tissue above, in which the algæ are embedded. These algæ (seen by crushing the section to be filaments of Scytonema even less altered than in Heppia) are, with few exceptions, pushed aside, and the uppermost layer of parallel hyphæ begins to take part in this active growth. But whereas in the deeper layers the area of growth is equally bounded on all sides by the thalline hyphæ, and has therefore no tendency to increase in one direction more than in another, the hyphæ forming the upper layers, when stimulated by the growth below, can grow freely upwards. Such a growth takes place, and the iso-diametric cells composing this layer begin to increase in length in a direction perpendicular to the surface. Thus these cells which here take the place of a true cortex are themselves transformed into a layer of upward-growing hyphæ, and, as we shall see, are to be considered as forming a subhymenial layer. (Plate II. Fig. 12.) We find accordingly the appearance of an inverted cone, the base composed of these metamorphosed superficial cells rising to a height above the normal surface of about 0.05 mm., the apex resting upon the dense layer forming the lower portion of the thallus. This condition of things is quite different from that seen before, where an existing cortex was ruptured by a hymenial disk arising beneath it. The parallel hyphæ forming the subhymenial layer upon the surface now begin to branch copiously, the branches arising parallel with the hyphæ

themselves, thus increasing very rapidly the area of the disk. But already this disk is elevated above the surface, and, being free to expand on all sides, spreads out in a fan-like manner, its edges encroaching more and more upon the thalline surface around it. Finally the hyphæ forming the central area of the disk give rise, from their tips, to elongated cells, which may at once grow into either asci or paraphyses, or may again bifurcate and produce the asci and paraphyses as a second order of branches. (Plate II. Figs. 13–16.)

The tips of the hyphæ, which, by the spreading out of the margin of the apothecium over the thallus, have come to be directed obliquely, or even perpendicularly, to the surface, elongate, and though I have been unable to see with certainty any definite anastomosis between these tips and the cells composing the thalline surface, they undoubtedly serve to attach the lower surface of the apothecium to the surface of the thallus. The extremely gelatinous character of the mature apothecium may be seen from the fact that when wet it becomes almost transparent, so that small foreign bodies on the surface of the thallus may be seen with a hand-lens through the tissue of the overspreading apothecium. This gelatinous quality renders it a matter of extreme difficulty to separate, sufficiently for exact study, the elements of the hymenium. With care and patience, however, it may be done. The asci are colored blue with any preparation of iodine, but no other part of the apothecium is so affected, nor at any stage in its growth, as far as my observation goes, does treatment with iodine bring to light any differentiated cells which might be mistaken for ascogonium, trichogyne, or ascogenous cells. The whole process of growth seems to be a purely vegetative one.

Pannaria rubiginosa, (Thunb.) Delis.

In the group of Pannaria species represented by *P. rubiginosa* we have a still nearer approach to the Collemaceous type. The anatomical structure of the thallus, while on the one hand, by reason of the presence of a thick cortex investing the upper surface and the absence of cortex on the lower surface, it seems to revert to the Peltigera type, seems on the other hand, from the nature of the algæ and the character impressed upon the thallus as a whole by that nature, to be differentiated widely from all preceding types. The algæ occupy nearly the full depth of the thallus, and at first sight present the appearance of irregular groups of small bluish-green cells embedded in spherical masses of jelly. By crushing a thin section, however, the cells composing these groups are seen to be arranged, not in straight filaments as in Heppia and *Pannaria molybdea*, but in curved chains

of small spherical cells provided here and there with definite heterocysts. Each of these chains is embedded in a definite, more or less spherical mass of jelly, and by any one familiar with the types of the lower algæ they are recognized as unaltered Nostoc colonies.

With the exception, then, of the fact that the hyphal nature of the thallus still preponderates slightly over that induced by the gelatinous sheaths of the Nostoc colonies, this lichen presents us with a structure identical with that seen in Leptogium.

It is a matter of very little difficulty to find the earliest stages of the reproductive organs, both spermogonia and apothecia. They are mostly limited to separate thalli, and are densely crowded, the apothecia particularly, occupying the centre of the lobe in large numbers, the edges being comparatively free from them. If then a series of sections be made progressively from the edge towards the centre, the different developmental stages can be clearly traced. It occasionally happens that near the edge of a thallus lobe, otherwise provided with apothecia only, there occur a very few spermogonia, and in such cases a comparison of the early stages of the two organs lying side by side in the same section is very instructive. Nothing further need be added here to the course of spermogonial development already described.

The apothecial primordia differ considerably from the youngest spermogonia. In size they are rather larger, in shape they are much more spherical, and in position they are more deeply sunk in the thallus, so that very few algal colonies occur below them. The structure and origin of the small, spherical masses of interwoven hyphæ forming the primordia are easily made out, and although it seemed highly probable, from the close relationship evidently existing between this lichen and the Collemaceæ, that here would be found some form of sexual reproduction analogous to that described by Stahl in Collema, I was unable to find any trace of such a condition. Before the simple character of the young apothecium has begun to give place to the more complex condition seen later, the thalline elements above it begin to disorganize. What this result is due to it is impossible to state. The growth of the primordium undoubtedly exerts a tension on the overlying tissue, but the disintegration seems rather to be caused primarily by the death of the protoplasmic contents of the cells, and the absorption of the membranes, in a manner similar to that which produces the central cavity in the young spermogonium. But from whatever cause, by the time the first paraphyses have arisen from the upper part of the primordium, the overlying tissue has disappeared, and the young hymenium, though still deeply sunken in the

thallus, is free above. The growth now becomes more rapid, the disk enlarges, and, at the same time growing upwards, rolls back the thalline elements, so that by the time the disk appears on the surface it is surrounded by a large thalline exciple. The treatment adopted in other cases shows that here also both asci and paraphyses arise from a common system of hyphæ forming the thin hypothecium. (Plate III. Figs. 20, 21.) It often happens that the disk enlarges very much after reaching the surface, and that the exciple is thereby rolled back upon the thallus. Thus the cortex enveloping the apothecium is bent back upon the cortex of the thalline surface, and the former, as in *P. molybdea*, puts out hair-like processes which soon form an intricate hyphal layer. (Plate III. Fig. 18.) There are thus formed beneath the mature apothecium, except at its centre, two layers of cortex separated from each other by a loose weft of hyphæ.

COLLEMEI.

In approaching the truly homœomeric groups of lichens, we realize at once that we are now on debatable ground, for it is this group which formed the basis for the theory of lichen-sexuality, the one group, so far, in which the conclusions reached by Stahl and his followers seem plausible. In the subfamily Eucollemei, as understood by Tuckerman,* we find five genera, of which the last, the peculiar genus Hydrothyria, Russell, will first occupy our attention. I take up the consideration of this lichen now, instead of later, because I cannot but regard the synoptical position which it at present holds as by no means a settled one. Writing upon this point Tuckerman himself says: "In this type (Hydrothyria), remarkable alike in its characters and its habitat, Collema, Ach., which we found to reach its extreme of development in the Leptogia of more recent authors, may be said now to revert evidently towards Pannaria, or even Peltigera." † Instead, however, of regarding this type as a reversion to a preceding type, it seems more fitting, for the present at least, to consider this form as following directly upon the Peltigerei and Pannariei, which in many points it most closely resembles, and as forming an additional link between the homœomeric and heteromeric forms, while inclining decidedly toward the latter. The habit of *Hydrothyria venosa*, Russ., the only known representative of the genus, although in some measure like that of Leptogium, seems to approach much more nearly that of

* Tuckerman, Synopsis of North American Lichens, p. 5.

† Tuckerman, Genera Lichenum, p. 102.

Nephroma. It is however more decidedly erect and foliaceous than that lichen, the broad, undulate, fan-like lobes being frequently 3 cm. wide, and contracted below into a stalk which serves to attach the plant to the substratum. Frequently several such fronds are found growing together in a dense tuft, the expanded portions floating freely in the water, and often they form a dense growth covering the rocky bottom of the brook in which they occur over an area several feet in extent. Although at times, when a frond is pressed down upon the rocky substratum, it puts out from its lower surface a growth of rhizoids by which it becomes attached to the rock, in its normal condition, as far as my observation goes, the frond floats erect in the water, attached only by the short stalk-like contraction of the base referred to above. At the point where this stalk expands into the frond it gives rise to a number of veins which, in a manner analogous to that seen in Peltigera, spread out over one surface of the thallus, seldom anastomosing, however, except where they reach the free edge of the thallus-lobe or frond.

It will be seen, then, that Hydrothyria, while resembling Leptogium superficially in its rich olive-brown color, presents on closer examination points, even of habit, which are in no degree analogous to that genus. This fact is emphasized when we come to study the thallus in detail.

In considering the structure of the thallus, I will dwell at greater length upon those points which are indicative of the synoptical position of this lichen, — the general character of the thallus, the presence or absence of a cortex, and the nature of the algæ forming the host. If we examine a radial section of the thallus, we find that its structure near the centre differs materially from that near the edge. In the former case the hyphæ are very large (5.6 μ to 7.3 μ in diameter), and in the lower part of the thallus maintain a comparatively regular course parallel to the surface of the thallus. Owing to their large size and the comparative regularity of their course, the individual hyphæ may often be traced to a considerable distance, the interweaving and branching being not such as to cause much complexity of structure. In fact, the structure of this layer is much like that seen in the lower part of the thallus of *Pannaria molybdea*. On the lower surface this layer is bounded by a cortex consisting of a single row of cells which are differentiated from the cells immediately above them by their thicker and slightly brownish walls. This simple structure of the cortex recalls a like condition seen in Nephroma. The central part of the thallus is occupied by the wide algal zone, composed of dense aggregations of small bluish-green cells, among which the hyphæ run in inextricable confusion. So irregular is their course as

they traverse the algal zone, that the section has almost the appearance of a parenchymatous tissue, in the midst of which lie the groups of gonidia with no apparent regularity. On the upper surface we find the thallus bounded by a simple cortex developed from the medullary hyphæ, and in no way distinguishable from that which covers the other surface. (Plate V. Fig. 31.) It is almost impossible, so dense is the tissue, to determine from sections what is the nature of the gonidia, or even whether they are unicellular or filamentous. On crushing the section the algæ are freed with some difficulty from the investing hyphæ, and appear unicellular. Their shape, however, is not at all that of the ordinary unicellular gonidia. They are extremely irregular in outline, and generally present one or more flattened surfaces, as though they had originally been members of a moniliform series. By exercising great care I was finally able to separate a few groups of these cells without their becoming entirely disintegrated, and they then appeared as filaments composed of three to seven cells very similar in size and arrangement to the gonidia of *Pannaria molybdea*, though much more distorted by reason of the greater density of the investing tissue. (Plate V. Fig. 33.) They are undoubtedly of the Scytonema type, a fact which, if the nature of the host is to be considered of any importance in determining the systematic position of a genus, would bring Hydrothyria into relation with Pannaria rather than with Leptogium.

Although the algæ are more or less restricted to a certain zone of the thallus, this zone is so wide that the thallus cannot certainly be called heteromeric, but seems to approach much more nearly the partially homœomeric character of the Pannaria thallus, while, on the other hand, it is not truly homœomeric if we consider Leptogium as a type of such a thallus, nor does it partake of the gelatinous character of the Collema thallus. Toward the edge of the thallus, even the slight regularity seen in the arrangement of the hyphæ in the older portions gradually disappears, and the margin of the thallus presents in section only a very intricate texture of delicate hyphæ, occupied throughout by the algæ. The veins present essentially the same structure throughout. The hyphæ composing them arise as branches of the medullary hyphæ, and, pursuing a regular radial course, soon become united into firm bundles. The individual hyphæ become considerably increased in size near the point where the bundle arises from the medulla of the contracted base of the thallus frond; thus the bundle itself encroaches upon the thalline elements lying between it and the surface, and the latter becomes elevated in the form of a ridge or vein. (Plate V. Fig. 31.) As we approach the edge, the bundles of hyphæ

become smaller, and the veins therefore are less prominent, until at the extreme edge the ramifications are very delicate and anastomose at various points.

The structure of the thallus, then, though less gelatinous in its quality than that of *Pannaria rubiginosa*, would bring Hydrothyria into more or less close relationship with that genus. The presence of a well-defined cortex investing both surfaces of the thallus would give to Hydrothyria a position in our system farther removed from Leptogium and Collema than is Peltigera, — a position more nearly approaching that occupied by Nephroma, — while the character of the gonidia, on the other hand, would bring it into relationship with Pannaria and Heppia. While, then, Hydrothyria is to be considered in no sense as Collemaceous in its type, it becomes a matter of great difficulty to say exactly where it should be placed. It would seem fair to consider it as a member of the Pannaria type serving to connect that genus with the Peltigerei.

The development of the fruit is not an easy matter to follow. Spermogonia have not as yet been known to occur, nor have I been able to discover any sign of such organs, though I have examined material collected at different seasons of the year and in many localities. Material collected near New Haven, Connecticut, in June, 1889, has proved the best for the study of apothecial development. On these specimens the fully developed fruit is rarely seen in quantity, nor in dried specimens is it easy to detect the young stages. In fresh, moist material, however, the young apothecia may be seen with a hand-lens occupying in considerable numbers the extreme edges of the expanded thallus lobes. The marginal position of the fruit is a rule to which I have as yet seen no undoubted exceptions. It is true that a mature apothecium is occasionally found at some distance from the margin, but by examining younger stages it will be readily seen that these have arisen at the bottom of a sinus such as frequently extends deeply into a thallus lobe, and that by the marginal growth of the thallus around and beyond the young apothecium, the latter comes to occupy a position considerably removed from the margin. The young apothecia usually arise at a point on the margin of the thallus where the fine ramifications of the veins meet and anastomose, and if sections be made at such points it is a mere matter of time and patience to find the youngest stages. These are at first observable only as a slight change in the ordinary thalline structure of the extreme margin. The hyphæ are seen to be even more densely interwoven than normally, the usually very thin margin has thereby become slightly swollen, and

the cortical cells, as well as those immediately beneath them, are seen to be colored brownish. These initial appearances would mean but little did they not later become more emphasized, and more evidently associated with apothecial development. The tip swells more noticeably, owing to the active branching and interweaving of the hyphæ, and becomes club-shaped or even subspherical in section, the cortex becomes in many cases dark brown in color, and the delicate branches composing the upper part of the complex mass of hyphæ which causes the swelling of the tip, exhibit at the central point a slight tendency toward a regular arrangement perpendicular to the surface. As yet there has appeared no definite hyphal coil in any part of the tissue under examination, nor have I been able to discover, either in the tissue or on the surface, any appearance of a trichogyne. The tendency toward a regular arrangement in the upper part of the primordial mass of hyphæ soon becomes more marked, and there now exists, just below the surface, a layer of short parallel threads, the young hymenium, springing from the dense tissue of the primordium, and still in genetic connection with the tissue above. The tip in section, owing to the expansion in area of the young hymenium, becomes less spherical and flatter, and the brown coloration, considerably less marked than before, is now limited to the cortex immediately above the young hymenium. The paraphyses attain a length of 25 μ to 30 μ, and then their rate of growth becomes much less rapid. The same partial cessation of growth does not however take place in the surrounding and overlying tissue, and the latter, forced upwards, becomes torn away from the paraphyses and forms above the disk an arched cavity.

At this, or at a slightly earlier stage, there are to be seen in the hypothecium certain cells, the origin and object of which I am at a loss at present to explain. They are differentiated from the surrounding cells by their greater size, more rounded shape, and oily or granular contents. Their arrangement, furthermore, seldom exhibits any degree of regularity. The tissue overlying the disk gradually begins to show signs of approaching dissolution. The brown color of the rind spreads to the tissue beneath, and the whole mass covering the young hymenium becomes yellow or brown. The later development is quite normal. The expansion of the disk ruptures the rind in a stellate manner, and as soon as it appears upon the surface the hymenium increases with such rapidity that the thallus is bent back upon itself.

The formation of an exciple is extremely simple. As soon as the cortex is ruptured, the paraphyses again increase rapidly in length, until their tips are level with the surface, and the cortex, instead of being

rolled back, gradually disappears over the whole disk. The exciple of the mature apothecium then is not elevated above the hymenium, and the apothecium itself resembles in cross-section a shallow cup, the sides of which are elevated above the thalline surface, but not above the hymenium. Carpologically therefore, as well as structurally, Hydrothyria presents a marked affinity to Pannaria. It not infrequently happens that a double apothecium is formed. Both surfaces of the thallus being often, from their upright position in the water, equally exposed to external influences, the tendencies are only slightly, if at all, in favor of apothecial development on one surface rather than on the other, so that the primordium may give rise to a hymenium on both surfaces, and we have as the result two apothecia on opposite surfaces of the thallus. (Plate V. Fig. 32.) This equalization of tendencies pervades the whole thallus, there being nothing in sections to distinguish accurately one surface from the other, except the venation and the occasional feeble and scanty growth of short, hair-like processes where one surface by chance comes in contact with the substratum.

By employing the same treatment as heretofore, the asci and paraphyses may be traced to their origin with comparative ease, owing to the non-gelatinous quality of the hymenium. (Plate IV. Figs. 26, 27.) Treatment with iodine stains the asci alone blue, and the presence of larger cells in the hypothecium is no longer visible. This disappearance of cells previously seen to exist may be explained in one of two ways. The primordium, and therefore the hypothecium, as has been shown, are formed by a copious branching of the medullary hyphæ, these branches being much finer than the hyphæ from which they arise. Now it is almost to be expected that before the dense tissue is fully formed, and while the branches are still arising, it might easily happen that portions of the medullary hyphæ would become enclosed in this forming tissue. They would remain visible until completely overgrown, when they would become compressed and distorted to such a degree as to be no longer recognizable. If however we choose to consider them as analogous to the Woronin's hypha of certain Ascomyceteæ, notwithstanding their lack of any regular arrangement, we must believe that we have to do here with a form of reproduction analogous to that seen in Xylaria, and said to be characterized by the disorganization and disappearance of the Woronin's hypha and the absence of a trichogyne. Following out this analogy, we are led to the conclusion reached by De Bary in the case of Xylaria, that "the ascogenous cells and hyphæ do not spring from a distinct ascogonium, but, like the paraphyses, from parts of the primordium, while the

ascogonium, unmistakably present as Woronin's hypha, perishes without taking part morphologically in the formation of asci." This would account for the presence of the larger cells in the primordial tissue, and their absence later, but I must confess that such an explanation seems to me decidedly strained, though perhaps no more so than in the case of Xylaria already mentioned.

The absence of a trichogyne in Hydrothyria is to my mind as certain as in the other lichens studied, although in all cases the statement rests upon a purely negative proof. If we crush thin sections of an apothecium in which the asci are still young, (the same treatment being employed as formerly,) the elements of the hymenium may be separated easily. The paraphyses then appear as delicate, filamentous threads, which are either simple or copiously branched, and frequently exhibit a form of anastomosis by means of a bridge-like connection. (Plate IV. Figs. 28–30.) Both asci and paraphyses arise from the same vegetative cells of the subhymenial layer, and exhibit the same mutual relationship as in the other forms studied. (Plate IV. Figs. 26, 27.)

In conclusion, then, Hydrothyria can be regarded in no sense as a transitional form between the true foliaceous lichens of the Pannaria type and the gelatinous Collemaceæ. The presence of a cortex investing both surfaces of the thallus, the non-gelatinous character of the thalline tissue, and the Scytonemoid gonidia, are facts which taken together would give to the genus an anomalous position in the neighborhood of Peltigera and Pannaria. If such a transitional form does occur, it must rather be looked for in the genera Pannaria and Physma, where the Scytonema type of gonidia has given place to the Nostoc type, and where the dense but large-celled hyphal tissue of Heppia, in which the hyphæ give the character to the thallus, has, by the necessity arising from a decidedly gelatinous host, become modified, so that now the gelatinous membranes of the algæ preponderate, and take an equal or greater share with the hyphæ in impressing a definite character upon the thalline structure. Furthermore, as already noted by Tuckerman,* no one can examine the two species of Physma without being at once struck by the remarkable similarity in thalline structure, on the one hand, between *Physma luridum* and *Pannaria rubiginosa*, and, on the other, between *Physma byrsæum*, (Mass.) Afzel., and the typical Collemaceæ. (Cf. Plate VI. Fig. 35, and Plate III. Fig. 22. Also Plate VI. Fig. 34, and Plate VII. Fig. 36.)

At this late date it hardly seems necessary to dwell at much length upon the method of reproduction of the true Collemaceæ. The clear-

* Tuckerman, Genera Lichenum, p. 57.

ness of Stahl's account, emphasized as it is by numerous figures, has seemed to preclude the necessity for further confirmation. It was not so with regard to the heteromeric lichens. Neither Stahl's observations on this point, nor those of his followers, seemed to me sufficiently definite to warrant their acceptation without further confirmation. I therefore attempted this task in those groups of lichens which exhibit the most marked affinities to the Collemaceæ. But failing to discover there the points which I had been led to expect might well be found, I hesitated about accepting without further proof even the clearness of Stahl's statements in regard to the Collemaceæ. I have therefore been led to attempt a confirmation of these statements satisfactory to myself, with the following result. I have examined specimens of *Physma Müller*i, Hepp. (*Collema myriococcum*, Ach.); *Collema chalazanum*, Ach. (*Lempholemma compactum*, Krb., *Physma compactum*, Mass.); *Collema pulposum*, Ach.; *Collema nigrescens*, (Huds.) Ach.; and *Leptogium myochroum*, (Ehrh., Schaer.) Tuck. (*L. saturninum*, Nyl., *Mallotium tomentosum*, Krb.); and although I have not carried my observations as far as did Stahl, I have established in all cases the essential point, — the existence within the thallus of a coiled ascogonium prolonged upwards in the form of a multicellular thread, the trichogyne, whose tip appears above the surface of the thallus.

Collema chalazanum, Ach.

In sections through a young apothecium of this lichen, identical according to Nylander with *Physma compactum*, Mass., it is by no means difficult to detect, generally close to the denser investing tissue of the apothecium near the base, one or more structures identical with those figured and described in this species by Stahl as trichogynes. (Plate VII. Fig. 36.) They are delicate septate threads no larger than the ordinary fungus-hyphæ, but at once distinguishable from them by the thickening of the septa, more marked near the surface of the thallus than below. I have never seen more than four such hyphæ in one section, and generally there are but one or two, nor in a thin section should we expect to find more than this, since, according to Stahl, at the base of one spermogonium arise only six or eight trichogynes in all. Inasmuch as these peculiar hyphæ originate in a system of larger cells occupying the base of the young fruit, and reach the surface only at some distance from it, it is very rarely that the plane of the section corresponds exactly to that of a trichogyne, thus making it possible to trace the continuous course of the latter from its point of origin up to the projecting tip. I was also unable to follow the process

of the transformation of the spermogonium into an apothecium, for the reason that in the specimens examined by me the characteristic structure of the spermogonia had almost entirely disappeared, and nothing but rather advanced stages of apothecial development were to be found. In the case of *Physma Müller*i, however, there could be seen, projecting from the sides of the upper part of the young apothecia, traces of sterigmata. (Plate VII. Fig. 37.) I found that treatment with potassic hydrate, a solution of iodine and potassic iodide, and subsequently with dilute nitric acid, gave the best results in these and the following species, in bringing out the course and structure of both hyphæ and trichogynes, the iodine coloring them a deep brown, while the nitric acid, without destroying this coloration, partially dissolves the investing gelatinous sheaths of the algæ, enabling us to see the hyphæ with much more distinctness.

The peculiar process of ascus formation at the base of the spermogonium, as seen by Stahl and hinted at in my own observations, was established by Stahl as characteristic of the genus Physma, Mass., represented by the species *P. franconicum*, *P. compactum*, and *P. myriococcum*. On other than carpologic grounds, however, recent writers have placed these species in the genus Collema, the first and second under the name *C. chalazanum*, the third as *C. myriococcum*, and even this form, it is stated, may prove to be identical with *C. chalazanum*.* Of the two remaining species of this old genus Physma,† *P. sanguinolentum*, Krmph., and *P. Mülleri*, Hepp, the former has been described by Koerber, but the description is not sufficiently definite to place the species with any certainty, although Koerber states that it resembles *Heppia adglutinata*, (Krmph.) Mass. [*H. Despreauxii*, (Mont.) Tuck.]. Specimens of *H. Mülleri*, Hepp, exist in the Hepp herbarium,‡ and I have been fortunate enough to be enabled to examine and compare the specimens. It is undoubtedly a Collema, very closely resembling in habit *C. myriococcum*, (Ach.) Arn., as well as *C. chalazanum*, Ach. The thalline structure agrees rather more closely with my specimens of the latter, while the spores are considerably smaller than the measurements given by Nylander for *C. chalazanum*, and agree with the authentic spore measurements of *C. myriococcum*. The spores of *P. Mülleri* measure 9.4–11.3 $\mu \times$ 7.5–9 μ, are uniseriate in the asci and round-elliptical in shape, thus agreeing exactly with the spores of *C. myriococcum*. We may then with considerable certainty consider

* Tuckerman, Synopsis of North American Lichens, p. 143.

† Koerber, Parerga Lichenum, p. 409.

‡ Lich. Helvet. Exsic. Schaerer and Hepp, No. 933.

Physma Müllerí to be a form of *Collema myriococcum*, leaving the identity of the latter species with *C. chalazanum* an open question. In examining specimens of *Physma Müllerí* I have found a type of reproduction identical with that seen in *C. chalazanum*, a spermogonium at the base of which arise ascogonic cells prolonged outside of the spermogonium in the form of trichogynes. (Plate VII. Fig. 37.) The septa of the trichogynes exhibiting a progressive process of thickening from above downwards, the sprouting of the ascogenous cells to form asci, the pushing aside and suppression of the sterigmata by the growing asci and paraphyses, and the final transformation of the spermogonium into an urceolate apothecium embedded in the thallus, are points which may be readily seen and followed in the species before us, and they serve still further to connect it with *C. chalazanum*.

We are now in a position to adopt one of two views. It has been shown that all the species of the genus Physma, Mass. (with the possible exception of *P. sanguinolentum* Krmph.) are, according to accepted methods of classification, to be grouped together as one or possibly two species of Collema. We find, however, that these two species present a distinct type of reproduction, essentially unlike that of many other species of Collema. We must therefore conclude, either that this is really a generic distinction, in which case the present genus Collema must be divided, or else that in the one genus we have at least two modifications of the sexual type of reproduction seen in certain of the Florideæ. The latter seems at present the more expedient. As yet the sexual reproduction of certain lichens has not been accepted as a basis of classification, and should it be so accepted certain species at least of Leptogium would have to come under Collema, while Collema would lose one or more species in favor of a new genus. A much more extended knowledge of the gelatinous lichens must exist before we can venture to state even that these two modifications of the type of reproduction are the only ones which exist in the Collemaceæ.

I have further followed Stahl's investigations upon certain species of Collema and Leptogium in which the spermogonia and apothecia are separate, and the ascogonium exists as a simple coiled hypha reaching the surface by means of a multicellular trichogyne, and, together with the neighboring hyphæ, developing directly into an apothecium. Among the lichens provided with a cortex I have examined only *Leptogium myochroum*, (Ehrh., Schaer.) Tuck., and it is only necessary to state that the points observed by me have been entirely confirmatory of the results obtained by Stahl from his study of this species and of *L. microscopicum*. Here again, in the face of Stahl's

accurate account, I do not consider it necessary to occupy time and space in describing the process in detail. The figure (Plate VIII. Fig. 38) represents with sufficient accuracy the structure and general appearance of the ascogonium and trichogyne. The same remarks apply to *Collema pulposum*, (Bernh.) Nyl., and *Collema nigrescens*, (Huds.) Ach. The latter gives peculiarly good results, showing that the stages of development from the formation of the ascogonium to that of the mature spermogonium agree exactly with those described by Stahl in *C. microphyllum*, Ach. Sections made near the margin of the thallus, which is generally beset with young apothecia, show large numbers of primordia consisting of a large-celled coil invested more or less closely by branches of the neighboring hyphæ, lying between the young apothecia. (Plate VIII. Fig. 41.) Coils not yet thus invested are more rarely seen. They do occur, however, and the following points may readily be established. (Plate VIII. Fig. 40.) With iodine they do not turn blue, but give a very pronounced protoplasmic reaction. They arise directly from an ordinary thallus hypha, form two or three spiral coils, and are then prolonged upwards in the form of a straight or curved multicellular trichogyne. The tip rises above the surface, swells considerably, and is cut off by a cross wall arising at the base of the swollen portion. I was unable to see the act of fertilization, or the subsequent changes in the trichogyne, but the later development corresponds exactly with that of *C. microphyllum*.

It is possible now to summarize our knowledge of the reproductive process in the Collemaceæ as follows, bearing in mind that this list includes all the species thus far studied, and only those: —

I. Type characterized by the transformation of the spermogonium into an apothecium after the fertilization of the carpogonium.

Physma franconicum, Mass. } = *Collema chalazanum*, Ach.
" *compactum*, Mass. }
" *myriococcum*, Mass. = *Collema myriococcum*, Ach.
" *Müller*i, Hepp, = *Collema myriococcum*, Ach.

II. Type characterized by complete separation, throughout their course of development of spermogonia and apothecia.

Leptogium Hildenbrandii, Nyl. }
" *Menziesii*, Nyl. }
" *saturninum*, Nyl. } = *Leptogium myochroum*,
Mallotium tomentosum, Krb. } (Ehrh., Schaer.) Tuck.
Collema tomentosum, Hoffm. }
" *saturninum*, (Dicks.) Ach. }

Collema microphyllum, Ach.
Synechoblastus conglomeratus, Krb. = *Collema conglomeratum*, Hoffm.
Collema multifidum, (Scop.) Krb.
" *pulposum*, (Bern.) Nyl.
" *nigrescens*, (Huds.) Ach.

The work for the future must be the careful examination of all forms of gelatinous lichens, and the accurate observation of all types of reproduction found to occur in the group.

Summary of Results.

1. My investigations upon the Collemaceous genera Leptogium and Collema, under which latter genus are to be included the forms described by Stahl under the name Physma, Mass., are entirely confirmatory of the results arrived at by Stahl in his investigations upon those groups. There exist in the Collemaceæ at least two modifications of a sexual type of reproduction, one monoclinic, of which *Collema chalazanum*, Ach., is a typical example, the other diclinic, exemplified by *Leptogium myochroum*, (Ehrh., Schaer.) Tuck., and *Collema nigrescens*, (Huds.) Ach.

2. The genus Hydrothyria, represented by *H. venosa* Russ., cannot, as heretofore, be considered as typically Collemaceous, but is to be regarded as transitional in its character, and related to the genera Peltigera and Pannaria, between which it forms a more or less definite link.

3. In the groups of typically heteromeric lichens more nearly related structurally to the Collemaceæ, as well as in the transitional forms represented by Pannaria, Heppia, and Hydrothyria, there exists, so far as I have seen, no visible evidence of any sexual form of reproduction. The development of the fruit is a purely vegetative process analogous to that seen in many Ascomycetous fungi.*

4. In all such lichens, as far as my observation goes, there exists at no stage in the development of the fruit any differentiation of the hyphæ into an ascogenous system and an enveloping system distinct from it.* Both asci and paraphyses arise from one and the same system of hyphæ, and with respect to their origin exhibit the closest

* Pleospora. Miyabe, "On the Life-History of Macrosporium parasiticum, Thüm.," Annals of Botany, Vol. III. No. IX. pp. 10, 24.

Claviceps, Epichloe, and Nectria. De Bary, Comparative Morphology and Biology of the Fungi, Mycetozoa, and Bacteria, English translation, p. 200.

Sphyridium, Cladonia, and Bæomyces. Krabbe, "Entwicklung, Sprossung und Theilung einiger Flechtenapothecien," Botanische Zeitung, 1882.

mutual relationship, thus presenting a marked analogy to those Ascomycetous fungi in which the fruit arises as the result of a purely vegetative process of hyphal growth.

In conclusion, I must express my thanks to Dr. W. G. Farlow who, during the course of my investigations, very kindly allowed me the free use of his herbarium and library.

EXPLANATION OF THE PLATES.*

PLATE I.

Figs. 1–3. *Sticta anthraspis*, Ach. Asci and paraphyses after treatment with iodine. × 500.

Figs. 4–6. *Sticta amplissima*, (Scop.) Mass. Asci and paraphyses after treatment with iodine. × 500.

Figs. 7, 8. *Peltigera polydactyla*, (Neck.) Hoffm. Asci and paraphyses after treatment with iodine. × 500.

Figs. 9–11. *Nephroma tomentosum*, (Hoffm.) Krb. Asci and paraphyses after treatment with iodine. × 500.

PLATE II.

Pannaria molybdea, (Pers.) Tuck.

Fig. 12. Cross-section of margin of thallus with immature apothecium. × 110. The outline is drawn with the camera, the detail being drawn free-hand.

Fig. 13. Portion of an older hymenium near the margin, with asci, paraphyses, and subhymenial tissue. Free-hand drawing, partially diagrammatic.

Figs. 14–16. Asci and paraphyses. × 500.

Fig. 17. Isolated gonidia. × 500.

PLATE III.

Pannaria rubiginosa, (Thunb.) Delis.

Fig. 18. Portion of immature apothecium. Free-hand drawing, partially diagrammatic.

Fig. 19. Isolated colonies of gonidia. × 500.

Figs. 20, 21. Asci and paraphyses, after treatment with a very dilute solution of iodine and potassic iodide. × 500.

Fig. 22. Cross-section of thallus. The outline drawn with the camera, the detail completed free-hand. × 140.

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* The drawings in all cases, unless otherwise stated, were made with the aid of the camera lucida. The paraphyses are colored darker by the iodine than the cells from which they arise.

PLATE IV.

Figs. 23–25. *Heppia Despreauxii*, (Mont.) Tuck. Asci and paraphyses. × 1100.
Figs. 26, 27. *Hydrothyria venosa*, Russ. Asci and paraphyses after treatment with iodine. × 500.
Figs. 28–30. *Hydrothyria venosa*, Russ. Young paraphyses with bridge-like connections. × 500.

PLATE V.

Hydrothyria venosa, Russ.

Fig. 31. Cross-section of vein and portion of thallus. × 140.
Fig. 32. Cross-section of double apothecium. Free-hand drawing.
Fig. 33. Isolated gonidia. × 1025.

PLATE VI.

Fig. 34. *Physma byrsæum*, (Afzel.). Cross-section of a thallus 0.9 mm. thick, showing only the upper and lower surfaces. × 140.
Fig. 35. *Physma luridum*, (Mont.). Cross-section of thallus. × 140.

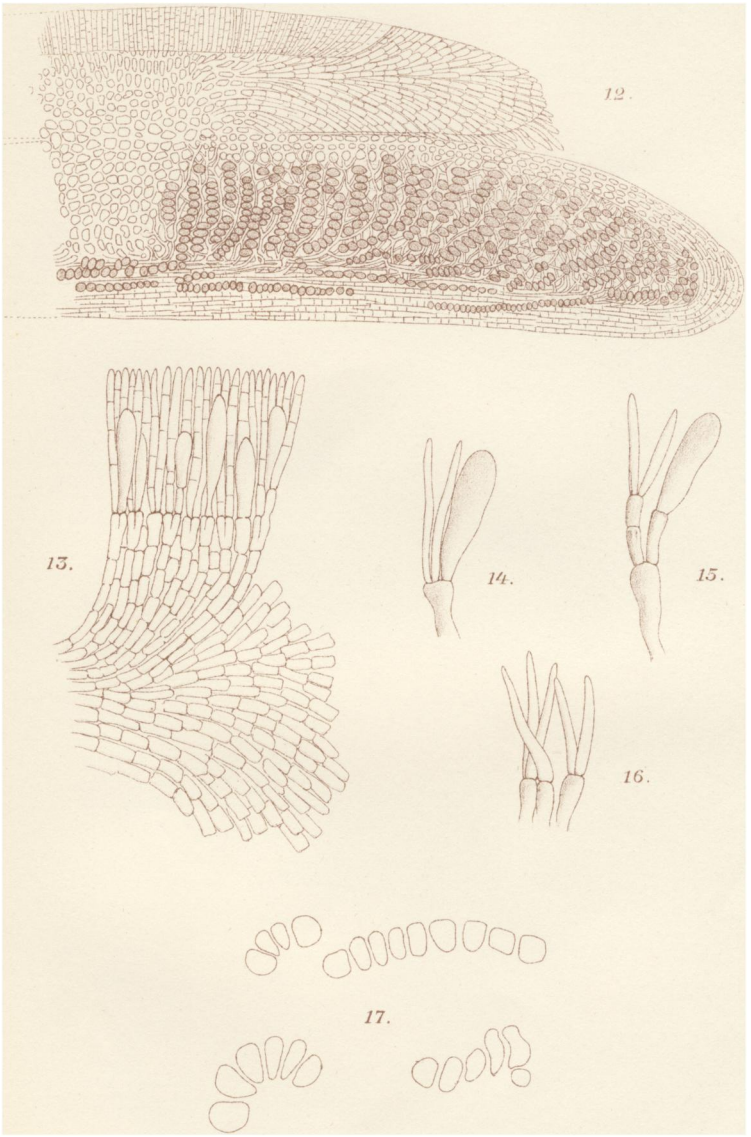
PLATE VII.

Fig. 36. *Collema chalazanum*, Ach. Cross-section of young apothecium, with trichogynes. × 140.
Fig. 37. *Physma Mülleri*, Hepp. Cross-section of young apothecium occupying a spermogonial cavity with portion of a trichogyne. × 140.

PLATE VIII.

Fig. 38. *Leptogium myochroum*, (Ehrh., Schaer.) Tuck. Ascogonium and portions of two trichogynes. × 500.
Fig. 39. *Collema pulposum*, (Bernh.) Nyl. Ascogonium. × 500.
Fig. 40. *Collema nigrescens*, (Huds.) Ach. Young ascogonia. × 500.
Fig. 41. *Collema nigrescens*, (Huds.) Ach. Older ascogonium partially enveloped by the surrounding hyphæ. The hyphæ which rise toward the upper surface of the thallus already show a definite parallel arrangement. × 500.

W. C. Sturgis, del. B. Meisel, lith.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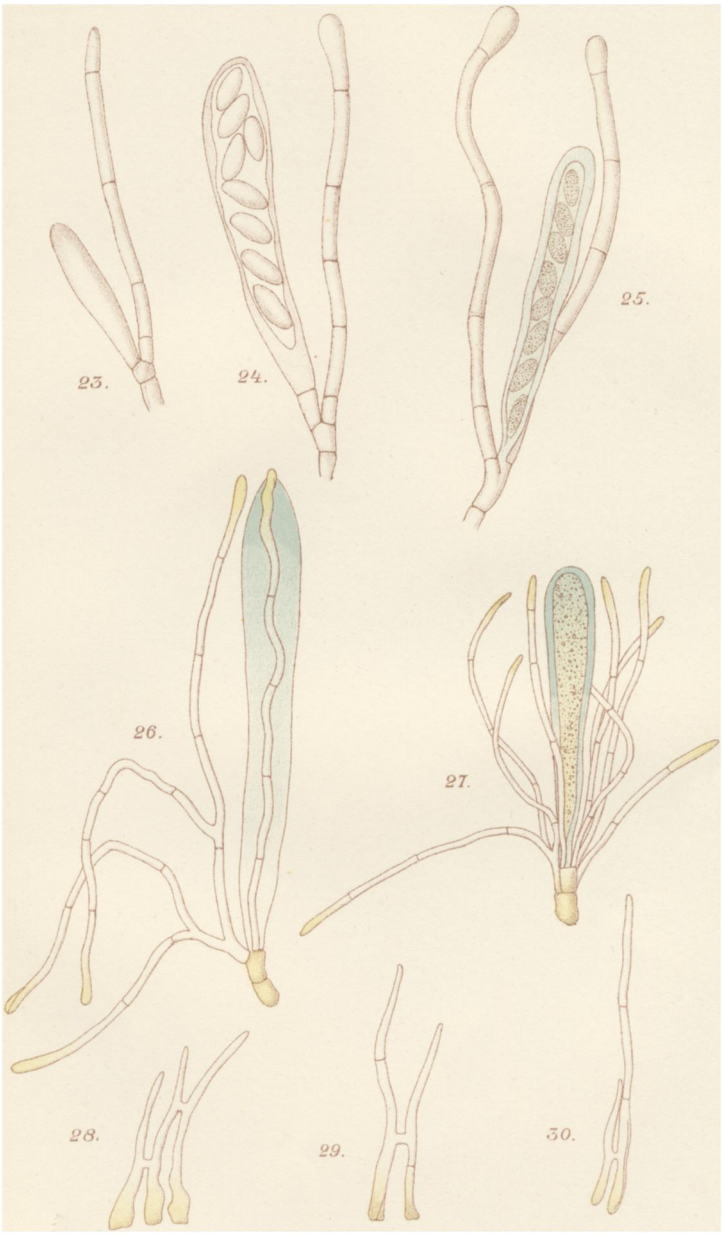
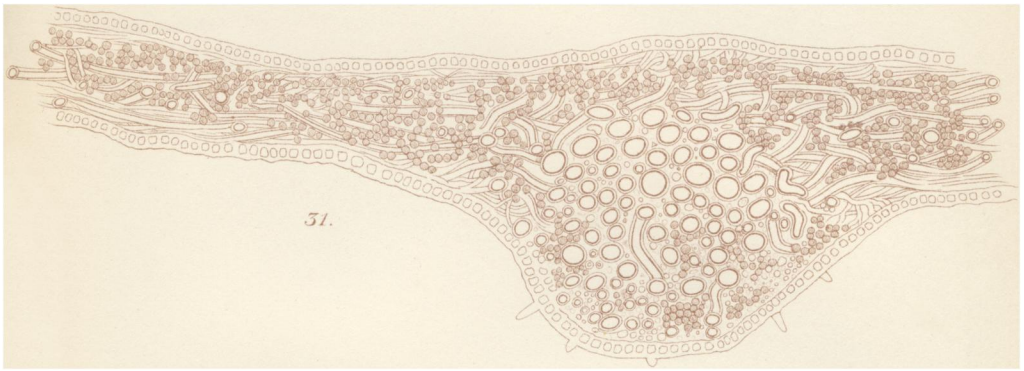
W. C. Sturgis, del. B.Meisel, lith.



W. C. Sturgis, del. B.Meisel, lith.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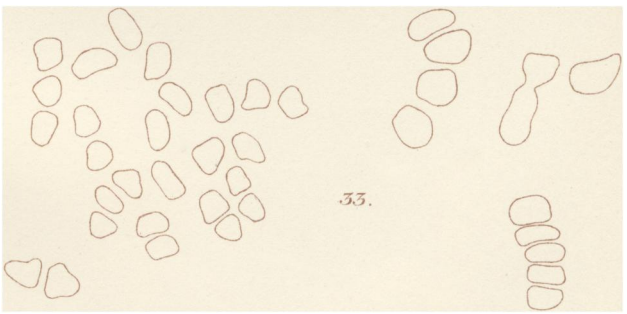
32.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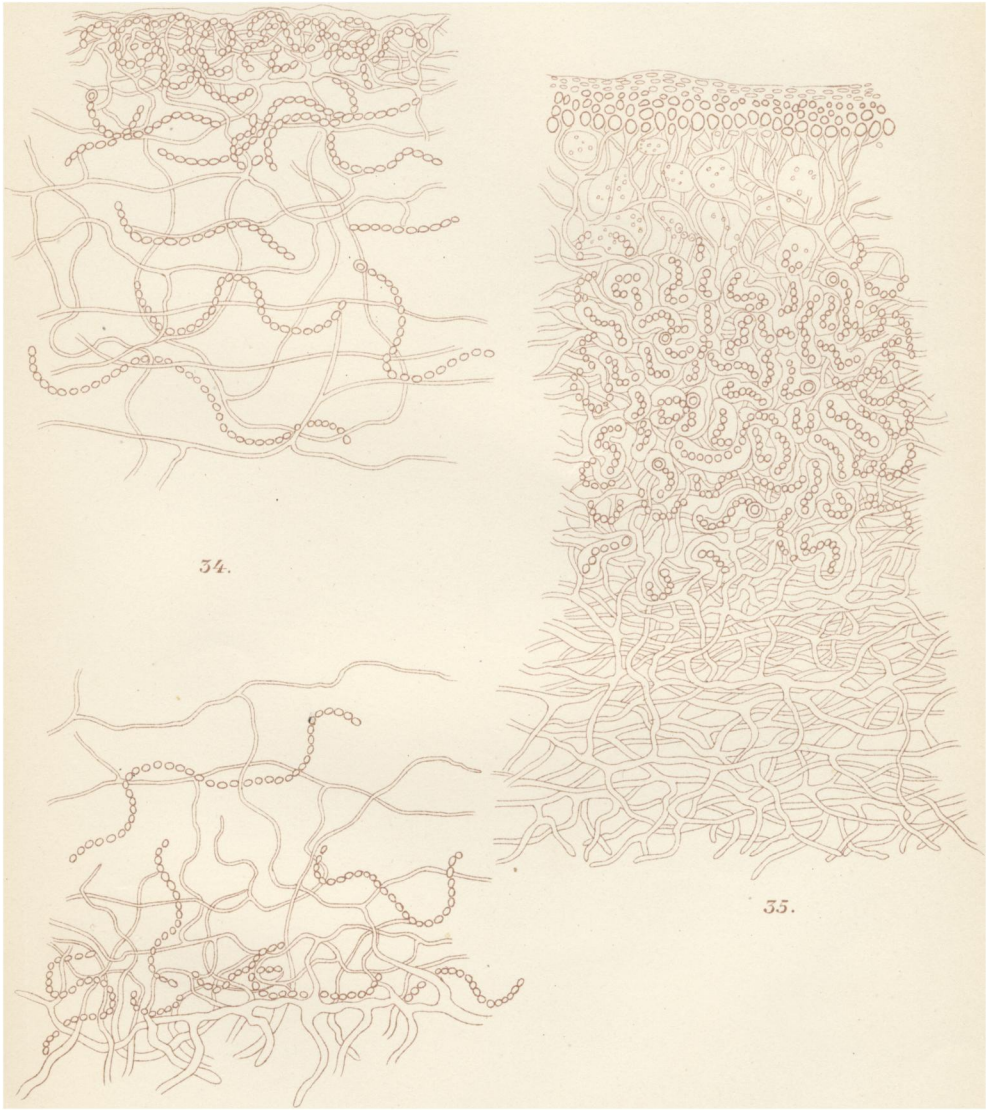

34.

35.

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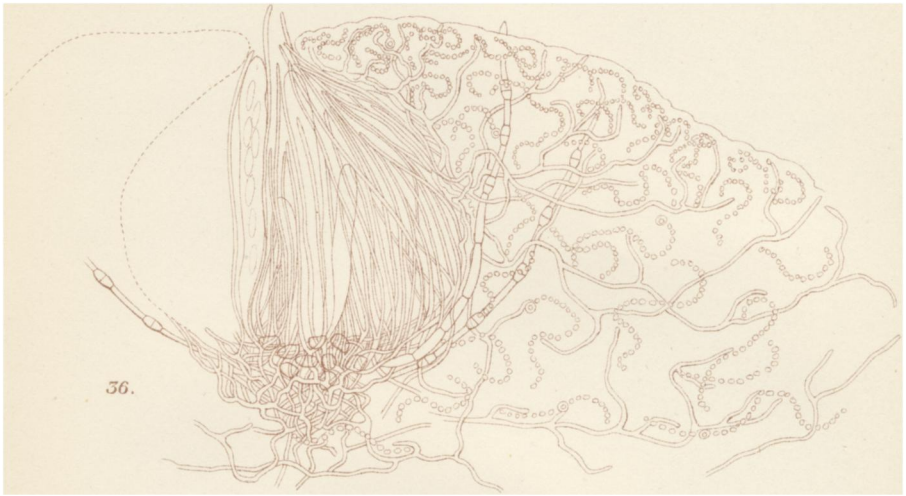
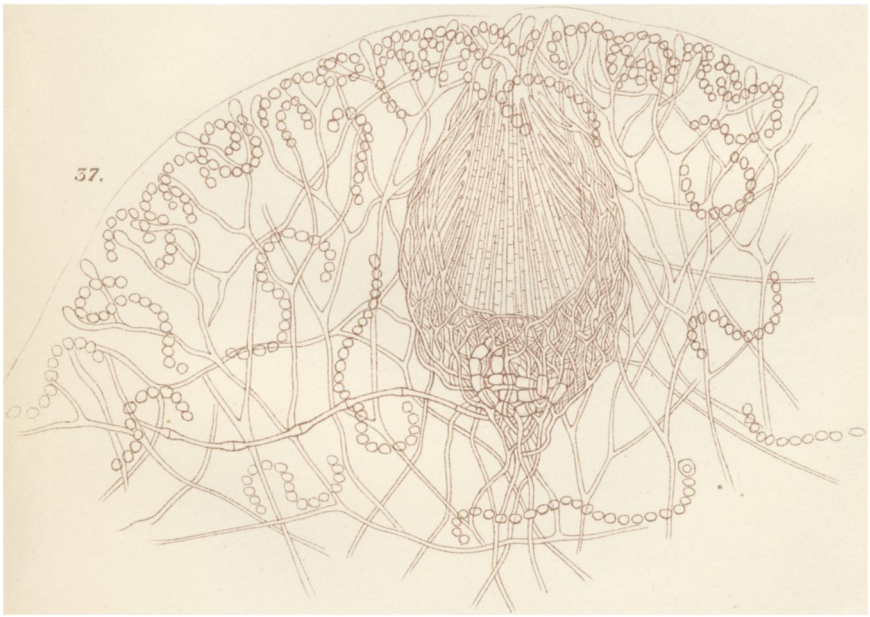
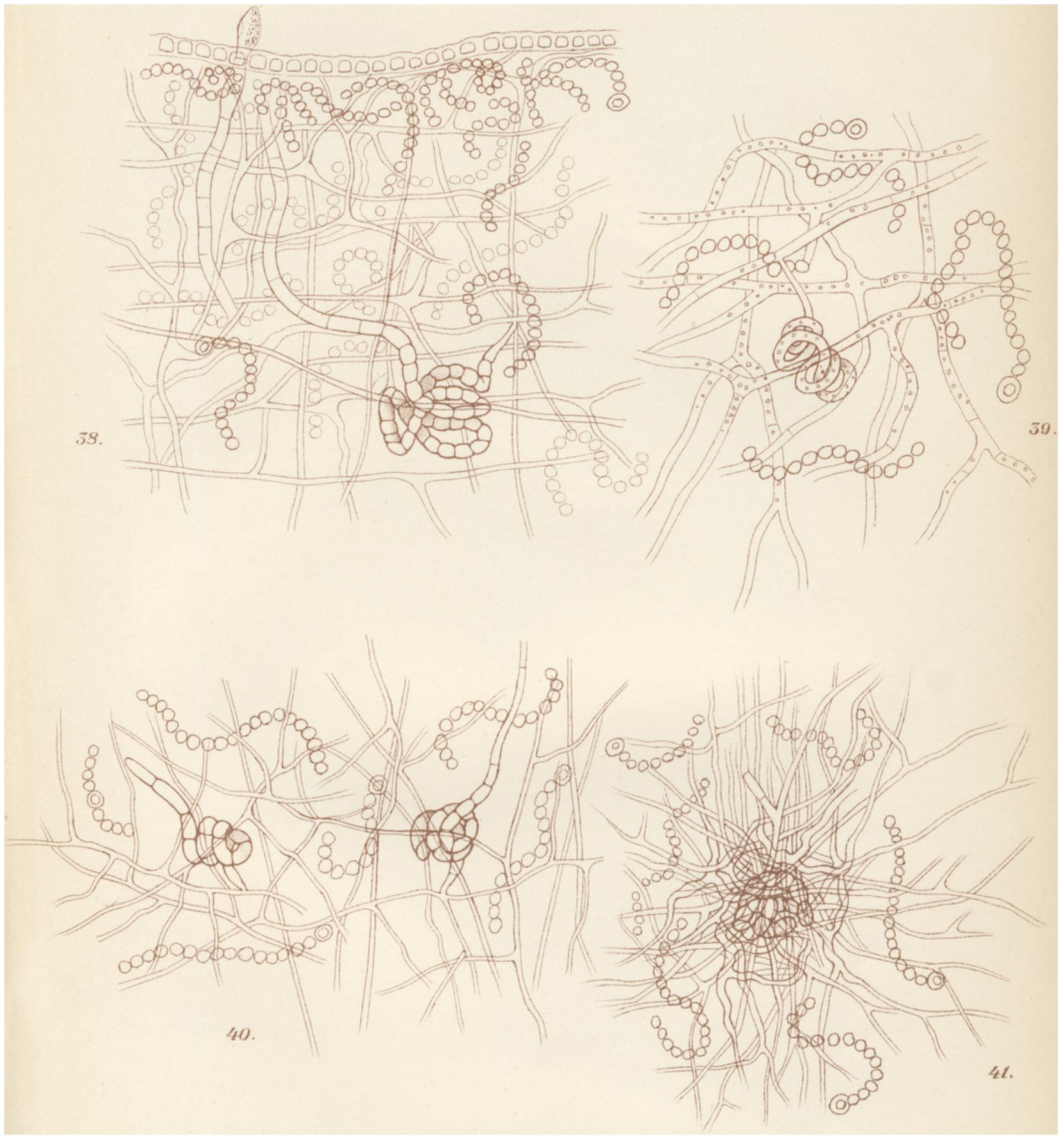




W. G. Sturgis, del. B.Meisel, lith.



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